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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61L 2/18 — A1

(11) International Publication Number: WO 94/00161

(43) International Publication Date: 6 January 1994 (06.01.94)

(21) International Application Number: PCT/US93/06096

(22) International Filing Date: 25 June 1993 (25.06.93)

(30) Priority data:
07/905,344 29 June 1992 (29.06.92) US

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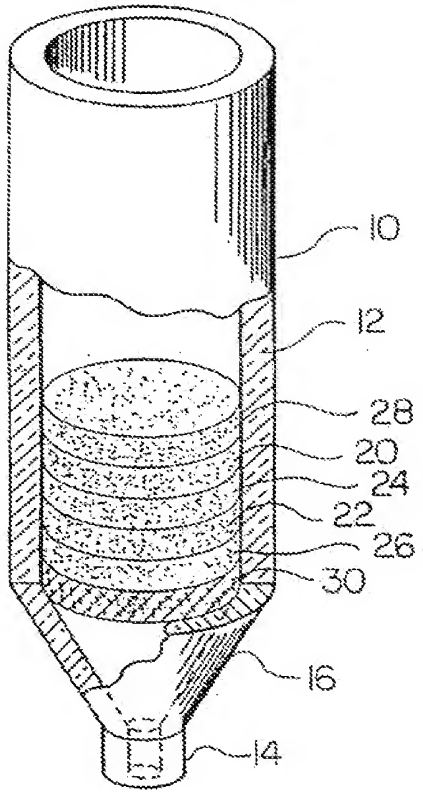
(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: POVIDONE HYDROGEN PEROXIDE PRESERVATION OF BLOOD, TISSUES AND BIOLOGICAL FLUIDS

(57) Abstract

The treatment and preservation of blood, blood derivatives and other body tissues, fluids and cells with PVP-H_2O_2 and then quenching the oxidizing potential of hydrogen peroxide in the PVP-H202 to kill pathogenic microbes without destroying the utility of the tissues, fluids, and cells is disclosed. Figure (1) depicts an apparatus (2) for contacting a liquid material with PVP-H_2O_2 and with either or both of (a) an hydrogen peroxide absorbing material and/or (b) a hydrogen peroxide reducing material, and for providing other materials for processing biological liquids, in particular, according to this invention.



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POVIDONE HYDROGEN PEROXIDE PRESERVATION OF BLOOD, TISSUES AND BIOLOGICAL FLUIDS

Field of the Invention

This invention relates to the treatment and preservation of blood and blood derivatives, the treatment and preparation of other body tissues and cells, the treatment and preparation of tissue cultures and tissue culture products, and the preparation of laboratory reagents, standards and samples. According to the invention, povidone hydrogen peroxide, PVP-H_2O_2, is used in the treatment of treating biological materials. Thereafter a physiologically compatible reducing agent such as an ascorbate salt, as an additive or on a solid support, e.g. in a bed or filter of solid povidone, may be used to remove the last traces of oxidizing iodine. This invention may, thus, be used to kill or inactivate virus, bacteria, chlamydia, rickettsia, mycoplasma and other potentially pathogenic microorganisms and to remove all oxidizing iodine.

The treatment and preparation of human blood, tissues, etc. and of the blood, tissues, etc. of other animals are contemplated. In general, the field of this invention lies in medicine and veterinary practice; most examples being related to the practice of medicine for the benefit of human patients, use in analogous fields of veterinary medicine to the extent applicable being within the scope of the invention.

Background of the Invention

Those who deal with blood and other invasively obtained body fluid samples risk infection from the samples. Those at risk include the doctor, nurse or clinical technician who takes the sample, the technicians who handle the sample and who use the sample in conducting analyses and tests, those who handle the sampling and testing equipment and apparatus, and the entire chain of individuals who attend to the disposal of sampling apparatus and the like, from the individuals who pick up the used apparatus through those who ultimately dispose of the apparatus, usually in specially designed high temperature furnaces. The risk is substantial, as evidenced by the fact that nearly all health care professionals with long experience carry the Epstein-Barr virus (EBV) and/or cytomegalovirus (CMV). Other pathogenic viruses to which health care workers, and those who handle blood and fluid sampling and handling apparatus, are exposed include

hepatitis and human immunodeficiency virus (HIV) as well as a large number of less life-threatening viruses.

Another organism which is frequently present in blood and blood products or fractions and which presents a serious risk in certain procedures is the bacteria *Yersinia enterocolitica* which is become a serious contaminant, surpassing *Salmonella* and *Campylobacter* as a cause of acute bacterial gastroenteritis. A significant increase in transfusion related infections of *Y. enterocolitica* has been reported, Tipple, et al., ***Transfusion*** 30, 3, p.207 (1990). *Y. enterocolitica* and other bacteria which propagate at relatively low temperatures, e.g. *Staphylococcus epidermidis* and *Legionella pneumophila*, present, potentially, a serious threat in blood products.

In addition to the risk of transmitting infectious disease via blood or blood products, the growth of bacteria in blood and blood products at various stages of production and processing introduces pyrogens into the blood component or product which must be removed before the product can be used in therapy. Introduction of povidone-hydrogen peroxide at an early stage in processing of blood products is believed to reduce or eliminate the pyrogen-load of the ultimate product or fraction.

Protozoa give rise to many diseases, some of great medical and economic importance. Examples of such protozoa are the genus *Plasmodium*, e.g. *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*, which causes malaria, *Trypanosoma*, which causes Chagas' disease, and *Leishmania*, which cause a variety of leishmaniasis. The method of this invention is effective in eliminating these causative organisms in blood and blood products.

Many viruses, in both animals and humans, may be transmitted by artificial insemination using sperm from infected individuals. Bovine leucosis (Mateva. V. et al, *Monatsh. Veterinaermed.* 1987, 42(9) 310) and bovine rhinotracheitis virus are transmitted by sperm of infected bulls. (Kupferschmied, H. U., et al *Theriogenology* 1986, 25(3) 439). Singh, E.L. (*10th Int. Cong. on Animal Repr. and Artificial Insemination, Cong. Proc. V. 1 - IV*, 1984) concluded that some viruses, e.g. bluetongue virus (BTV), infectious bovine rhinotracheitis virus (IBRV), bovine viral diarrhea virus (BVDV), foot and mouth virus (FMDV), akabane virus (AV) and bovine parvovirus (BPVP), were transmitted via seminal fluid rather than in the sperm cell.

Generally, this invention is applicable to the treatment of donated blood and products produced from blood, tissues and fluids for inactivating virus, bacteria, chlamydia, rickettsia, mycoplasma and other potentially pathogenic microorganisms.

Among the important potential pathogens to which this invention is applicable is cytomegalovirus (CMV). Herpesviruses, of which CMV is a member, represent a very large group of viruses which are responsible for, or involved in, cold sores, shingles, a venereal disease, mononucleosis, eye infections, birth defects and probably several cancers. The present invention is also useful in preventing the transmission of human immunodeficiency virus (HIV). While testing has made blood products safer than it was a decade ago, the complete elimination of HIV contaminated blood and blood products has not been possible using present knowledge and technology.

Blood plasma is used in the production of many important blood fractions, components and products. Transfusion plasma, per se, is frequently prepared as a single blood bag product; however, many plasma fractions and products are produced from large pools of plasma. There is a real and serious risk of infection to the technicians who handle individual blood bags and serum bags, and the risk of infection is multiplied many times in the handling of pooled plasma. There is, of course, a serious risk that the recipient of plasma or a plasma fraction or product may be infected unless suitable steps are taken to kill or inactivate potentially pathogenic organisms. Such steps are usually taken far down the chain of processing steps and frequently as the final step before use, storage or lyophilization, according to the product.

The production of pyrogens in plasma and plasma products during initial handling or handling down-stream in the process chain by the propagation of organisms which, at a later stage in processing, are inactivated or killed constitutes a serious problem to producers of plasma fractions and products. Pyrogen production could be eliminated or substantially reduced if pyrogen producing organisms were killed early in the process, e.g. in the initial whole blood or in the pooled plasma.

Virus infections, among the most serious being hepatitis, present a constant and serious risk to both handlers and recipients of blood and blood products. It has been shown that fractionation workers, particularly those engaged in the

preparation of plasma pools, are at high risk of developing hepatitis B. The high risk products are fibrinogen, AHF, and prothrombin complex. The low risk products are ISG, PPF, and albumin. The lack of infectivity of PPF and albumin is attributable to heating the final products at 60°C. for 10 hours; however, such process steps tend to denature certain products and are unsuitable in the preparation of heat sensitive products.

The risks of infection from whole blood are well-known. One of the great tragedies of modern medicine is the infection of many patients, most frequently hemophiliacs who require frequent blood transfusions, with HIV. The purification of the nation's and the world's whole blood for transfusion would constitute a monumental step forward in the history of medicine. The risks of infection from red blood cell concentrates is similar to comparable risks associated with whole blood.

As used here, the term "blood" means whole blood and blood fractions, components, and products of blood, unless "whole blood" or a specific blood derivative, e.g. a blood fraction, component or product of blood is stated. Thus, the term "blood" may apply to whole blood at the time of collection or a blood derivative at any stage in processing, as indicated by context. Blood derivatives mean blood components such as blood cell concentrates (red blood cells, platelets, etc.), plasma, and serum and products and factors prepared from blood such as albumin and the blood factors. Body tissues and cells means any tissue(s), organ(s) or cells or fluids which contain tissue(s), organ(s) or cells of animal origin. Thus, in a broad sense, body tissues and cells include blood and the cellular components of blood; however, for the most part, simply for clarity in presentation, blood is treated as a separate application of the invention. Examples of body tissues and cells include sperm, bone marrow, kidneys, cornea, heart valves, tendons, ligaments, skin, homograft or xenograft implants and prosthesis generally. Tissue and cell cultures means cells and tissues grown or enhanced in culture media and the culture media per se, but not including nutrients intended for use in cell cultures. Examples of a cultured tissue is cultured skin tissue for use in burn victims, cells and cellular products prepared by standard biological and/or genetic engineering techniques are other examples of tissue cultures. Laboratory reagents and standards, as used in this specification and the claims, means reagents and standards produced from or comprising human or animal fluids, cells or

tissues. Examples of such products are red blood cell panel utilized for typing blood, control sera and chemistry controls. Samples of tissues and fluids to be tested include samples of blood, urine, sputum, cell smears, etc. While the term "donor" is not usually applied to the individual from whom such samples are acquired, that term, "donor" will be used here in a more general sense to include the individual from whom any blood, tissue, cells or fluid is obtained for any purpose, and such term will be used to refer even to an unwilling donor.

If a tissue is explanted into the culture media for the purpose of propagating its cells, the procedure is called tissue culture whereas the explanting of individual cells into culture media would be called cell culture; however, both procedures are often referred to by the term "tissue culture" procedures without differentiation, unless the distinction is critical for some ancillary reason. This general usage of the term is employed here.

Tissue cultured cells are extremely fragile in many ways, having exacting requirements not only as to nutrients but also to the amount and type of resident organisms which can be tolerated, and culture media are highly susceptible to bacterial and/or viral infection.

Hydrogen peroxide (H_2O_2), mol wt 34.016, is a weakly acidic, clear colorless liquid, miscible with water in all proportions. The four atoms are covalently bound in a nonpolar H--O--O--H structure. It is now prepared primarily by anthraquinone autoxidation processes. It is used widely to prepare other peroxygen compounds and as a nonpolluting oxidizing agent. The reactions of hydrogen peroxide are:

Decomposition:	$2H_2O_2 \rightarrow 2H_2O + O_2$
Molecular additions:	$H_2O_2 + Y \rightarrow Y.H_2O_2$
Substitutions:	$H_2O_2 + RX \rightarrow ROOH + HX$
	$H_2O_2 + 2\ RX \rightarrow ROOR + 2\ HX$
Oxidations:	$H_2O_2 + W \rightarrow WO + H_2O$
Reductions:	$H_2O_2 + Z \rightarrow ZH_2 + O_2$

Hydrogen peroxide may react directly or after it has first ionized or dissociated into free radicals. In many cases, the reaction mechanism is extremely complex and may involve catalysis or be dependent upon the reaction environment.

Hydrogen peroxide can form free radicals by homolytic cleavage of either an O--H bond or the O--O bond.

$HOOH \rightarrow H. + .OOH$ (380 Kj/mol or 90 kcal/mol)

$HOOH \rightarrow 2.OH$ (210 Kj/mol or 50 kcal/mol)

The last equation predominates in uncatalyzed vapor-phase decomposition and photochemically initiated reactions. In catalytic reactions, especially in solution, the nature of the reactants determines which reaction is predominant.

Hydrogen peroxide is a strong oxidant and most of its uses and those of its derivatives depend on this property. It oxidizes a wide variety of organic and inorganic compounds, ranging from iodide ions to color bodies of unknown structure in cellulosic fibers. Hydrogen peroxide reduces stronger oxidizing agents such as chlorine.

Aqueous hydrogen peroxide is sold in grades ranging from 3 to 98%, mainly containing 35, 50, 70, or 90% H_2O_2. The 3-6% H_2O_2 solutions for cosmetic and medicinal use are obtained by diluting a more concentrated grade, usually with the addition of extra stabilizer. There is a USP specification for 3% H_2O_2.

Hydrogen peroxide is irritating to the skin, eyes, and mucous membranes. However, low concentrations (3-6%) are used in medicinal and cosmetic applications.

Hydrogen peroxide is used to treat wastewaters and sewage effluents, and to control hydrogen sulfide generated by the anaerobic reaction of raw sewage in sewer lines or collection points. It has been proposed as a supplemental oxygen source for overloaded activated sludge plants. It reportedly controls denitrification in secondary clarifiers and improves bulking conditions. It has been used as a flotation assistant. It has been generated in a wastewater reservoir by the cathodic reduction of oxygen.

Hydrogen peroxide has been used with povidone iodine in biocidal processes. A number of such methods are described in the following patents to Witkin, et al.

Simon, Gilbert I; Witkin, Roy T, US Patent 4997625 910305, describe the treatment of dental and medical instruments and appliances to be chemically sterilized by immersion in an admixture of an iodophor such as the povidone iodine complex or a quaternary ammonium compound such as cetyl pyridinium chloride and a peroxide such as H_2O_2, the antimicrobial action of the iodine derived from the iodophor being enhanced or potentiated by oxygen released from

the peroxide. The invention is applicable also to the chemical sterilization of surgical sites.

Witkin, Roy T, US Patent 4935248 900619, describes applying, spraying, or sponging antimicrobially effective amounts of aqueous iodophor peroxide solutions to the udder surfaces of cows, or other animals to be milked prior to milking. The udders are shampooed with a nonstaining shampoo containing iodine in a povidone-iodine complex and hydrogen peroxide as a nascent oxygen source.

Simon, Gilbert I; Witkin, Roy T, US Patent 4738840 880419, US Patent 4592489 860603, and US Patent 4567036 860128, describe pre- and post-operative dental and surgical procedures in and on structures and areas of the oral cavity to maintain sterility by the application of an antimicrobially enhanced aqueous solution of an iodophor constituting a source of iodine and a peroxide as a source of oxygen. The iodophor is preferably a povidone iodine complex soluble in water and the peroxide is preferably hydrogen peroxide, the oxygen from the peroxide acting to enhance the antimicrobial activity of the iodine derived from the povidone iodine complex.

Witkin, Roy T, US Patent 5066497 911119, discloses an antimicrobial veterinary composition having enhanced antimicrobial activity against a broad spectrum of microorganisms afflicting small and large animals. The composition is a solution or mixture povidone iodine complex and nascent oxygen obtained from a peroxide source.

Hydrogen peroxide has been combined directly with povidone to provide a dry powder source of H_2O_2.

Garelick Paul; Login, Robert B; Merianos, John J, US Patent 5066488 911119, describe a semi-anhydrous, suspension process for preparing substantially anhydrous complexes of PVP and H_2O_2 containing about 18% to about 22% by weight H_2O_2. The process comprises suspending substantially anhydrous PVP and an aqueous solution of 70 to 85% H_2O_2 in an anhydrous ethyl acetate medium to precipitate a free-flowing, fine white powders of the complex, and filtering and drying under vacuum at about 40-50°C. to form the desired product.

Lieberman, Herbert A; Login, Robert B; Merianos, John J, US Patent 5008106 910416, describe a method of using the product referred to by Garelick, et al, ibid, for reducing the microbial content of surfaces which comprises

contacting said surface with a microbiocidal amount of a substantially anhydrous complex of PVP and H_2O_2.

Biss Russell B; Cohen Jeffrey; Merianos John J; Taylor Paul D, US Patent 5077047 911231, also describe a process for the production of pvp-H_2O_2 products in the form of free-flowing powders. A fluidized bed maintained at a reaction temperature of about room temperature to 60°C. is contacted with finely divided droplets of a 30 to 85% by weight aqueous H_2O_2 solution. A 50-70% H_2O_2 solution is used, and the feed rate of introduction of the H_2O_2 solution is about 5-50 g/minute/kg PVP present. The pvp-H_2O_2 product preferably contains about 15-24%, preferably 18-22%, H_2O_2 (1:1 molar ratio) and less than about 5% water.

The important role of hydrogen peroxide as produced *in vivo* in the tissues and fluids of living mammals to kill invading pathogens has been extensively studied. See, e.g.: Sattar S.A.; Springthorpe *V.S., Rev. Infect. Dis. (USA), 1991, 13/3 (430-447) (Abstract)*; Effects of topical antimicrobial agents on the human neutrophil respiratory burst, Hansbrough JF; Zapata-Sirvent RL; Cooper ML, *Arch Surg May 1991, 126 (5) p603-8 (abstract)*; Depression of hydrogen peroxide dependent killing of schistosomula in-vitro by peritoneal exudate cells from schistosoma-mansoni infected mice, Smith J M; Mkoji G M; Prichard R K, *Am J Trop Med Hyg 40 (2), 1989, 186-194 (abstract)*; Oxidative and nonoxidative killing of actinobacillus-actinomycetemcomitans by human neutrophils, Miyasaki K T; Wilson M E; Brunetti A J; Genco R J, *Infect Immun 53 (1), 1986, 154-160 (abstract)*; Killing of actinobacillus-actinomycetemcomitans by the human neutrophil myeloperoxidase-hydrogen peroxide-chloride system, Miyasoki K T; Wilson M E; Genco R J, *Infect Immun 53 (1), 1986, 161-165 (abstract)*; Phagocytes use oxygen to kill bacteria, Baggiolini M, *Experientia Sep 15 1984, 40 (9) p906-9 (abstract)*; Regulation of membrane peroxidation in health and disease, Boxer LA; Harris RE; Baehner RL, *Pediatrics Nov 1979, 64 (5 Pt 2 Suppl) p713-8 (abstract)*; Oxygen metabolism and the microbicidal activity of macrophages, Johnston RB Jr, *Fed Proc Nov 1978, 37 (13) p2759-64 (abstract)*; Superoxide radical and the bactericidal action of phagocytes, Fridovich I, *N Engl J Med, Mar 14 1974, 290 (11) p624-5, (abstract)*; Relationship between extracellular stimulation of intracellular killing and oxygen-dependent microbicidal systems of monocytes, Leigh P C J; Nathan C F; Van Den Barselaar M T; Van Furth R, *Infect Immun 47 (2), 1985, 502-507 (abstract)*; Safety and immunogenicity of hydrogen

peroxide-inactivated pertussis toxoid in 18-month-old children, Siber G.R.; Thakrar N.; Yancey B.A.; Herzog L.; Todd C.; Cohen N.; Sekura, R.D.; Lowe C.U; *Vaccine (United Kingdom) , 1991, 9/10 (735-740) (abstract)*; In vitro studies of water activity and bacterial growth inhibition of sucrose-polyethylene glycol 400-hydrogen peroxide and xylose-polyethylene glycol 400-hydrogen peroxide pastes used to treat infected wounds, Ambrose U.; Middleton K.; Seal D., *Antimicrob. Agents Chemother. (USA) , 1991, 35/9 (1799-1803) (abstract)*; Bacteriolysis is inhibited by hydrogen peroxide and by proteases, Ginsburg I., *Agents Actopms (Switzerland) , 1989, 28/3-4 (238-242) (abstract)*; Characterization of hydrogen peroxide-potentiating factor, a lymphokine that increases the capacity of human monocytes and monocyte-like cell lines to produce hydrogen peroxide: Gately C.L.; Wahl S.M.; Oppenheim J.J., *J. Immunol. (USA) , 1983, 131:6 (2853-2858) (abstract)*; Control of the microbial flora of the vagina by H_2O_2-generating lactobacilli, Klebanoff, S.J.; Hillier, S.L.; Eschenbach, D.A.; Waltersdorph, A.M., *Journal of Infectious Diseases, v164 n1, p94(7), July, 1991 (abstract)*.

A comprehensive review of oxygen based therapies, including both ozone and hydrogen peroxide therapies, has been published; "Do oxygen therapies work? -- Claims for oxygen as a miracle element that can cure even AIDS are hotly debated," Thomson, Bill, *East West, v19, n9, p70(9), Sept, 1989*. Thomson refers to McCabe, OXYGEN THERAPIES: A NEW WAY OF APPROACHING DISEASE, (Energy Publications, 1989) for an extensive review of medical uses of ozone and hydrogen peroxide therapy. Some physicians are reportedly gaining some confidence in the therapeutic value of hydrogen peroxide, at least to the extent that some doctors are encouraged to administer hydrogen peroxide intravenously. Hydrogen peroxide has been reported in scientific literature as being used with "varying degrees of success" in the treatment of cardiovascular, pulmonary, and infectious diseases; immune disorders; and other illnesses such as Alzheimer's, cancers of the blood and lymph, and migraine headaches. Skeptics are , however, suspicious when a single remedy is claimed to cure many illnesses. The data regarding use of hydrogen peroxide intravenously appears to reach far back, at least to 1920 when T.H. Oliver and his associate researchers reported in The Lancet that thirteen out of twenty-five patients in Busrah, India who were given hydrogen peroxide intravenously fully recovered from influenzal pneumonia,

a disease that had become epidemic and had reached 80 percent mortality. Most doctors are skeptical of the largely anecdotal reports of hydrogen peroxide therapy without scientific controls and many regard the practice as quackery.

Shenep, J.L, et al, in a fairly recent study, reported a lack of antibacterial activity after intravenous hydrogen peroxide infusion in experimental Escherichia coli sepsis. Shenep J.L.; Stokes D.C.; Hughes W.T., *Infect. Immun. (USA), 1985, 48/3 (607-610) (abstract).*

Hydrogen peroxide has also been evaluated as a bactericide in poultry chilling water to kill bacteria that reside on the carcasses of poultry prepared for use as a food, Lillard H S; Thomson J E, *J Food Sci 48 (1), 1983, 125-126 (abstract).* While bacterial kills could be obtained, the poultry carcasses were degraded to the point where they would be unacceptable in the marketplace.

The oxidative degradation of tissues both *in vivo* and *in vitro* as well as the pain and discomfort resulting from contact of hydrogen peroxide with open wounds and membranes has discouraged workers from using hydrogen peroxide in all but a very limited number of applications. Oral ingestion of hydrogen peroxide has been tried with moderate to severe side reactions, e.g. nausea, vomiting, diarrhea and physical discomfort and with doubtful benefits, see Thomson, *supra.*

The questionable results from intravenously and orally administered hydrogen peroxide militates against the use of hydrogen peroxide in any circumstance where fragile cells or tissues would be contacted by this strong irritant-oxidant unless the need for a strong, direct contact biocide outweighs all other considerations, as is the case in some infected wounds or infected surgical incisions.

It has been determined, notwithstanding the mountain of discouraging evidence, that povidone hydrogen peroxide (PVP-H_2O_2) that can be used effectively to kill pathogenic microbes in blood, blood cell concentrates, blood fractions and other biological materials in accordance with the present invention.

Povidone is used generally to describe compounds described in the U.S. Pharmacopeia to identify polyvinyl pyrrolidone suitable for use in physiologically acceptable solutions and to include polyvinylpyrrolidone (PVP) compositions that have not yet been approved for use in the preparation of therapeutic compounds, and equivalents, as described hereinbefore. When percent concentrations are referred to in connection with povidone-hydrogen peroxide, the percentage refers

to the percent of povidone-hydrogen peroxide by weight, based upon the weight of the solution or material to which the povidone-hydrogen peroxide is added. Thus, a 1 weight percent (w/o) solution of povidone-hydrogen peroxide indicates that enough povidone-hydrogen peroxide has been dissolved to result in a concentration of 1w/o povidone-hydrogen peroxide. The ratio of polyvinyl pyrrolidone to hydrogen peroxide in the povidone-hydrogen peroxide product according to this invention is in the range of 1 part of hydrogen peroxide to 5-12 parts of povidone. Higher povidone to hydrogen peroxide are preferred in some instances, as described hereinafter. Typical stock solutions are 10% PVP-H_2O_2 (5,000 ppm to 20,000 ppm H_2O_2), 5% (2,500 ppm to 10,000 ppm H_2O_2) and 1% (500 ppm to 1,000 ppm H_2O_2). In those instances in which a povidone to hydrogen peroxide ratio of higher than about 12 to 1 is referred to, additional povidone (polyvinyl pyrrolidone) is added to increase the PVP to H_2O_2 ratio. The concentration of povidone-hydrogen peroxide in such compositions means the concentration of standard PVP-H_2O_2 (calculated as having an 0.05 to 0.2 parts of H_2O_2 to 1 part of PVP to H_2O_2 ratio, whether or not added in that ratio. PVP in excess is treated, for purposes of calculation, separately from the PVP in "standard" povidone hydrogen peroxide.

PVP-H_2O_2-PVP is used as an abbreviation for povidone enriched povidone hydrogen peroxide, i.e a composition in which the total povidone to hydrogen peroxide ratio is greater than 15 to 1.

PVP-H_2O_2-PVPLMW is used as an abbreviation for PVP-H_2O_2-PVP in which at least ten percent of the povidone has a molecular weight of no greater than approximately 12,000 daltons.

PVPXL-H_2O_2 is used as an abbreviation for solid, e.g. cross-linked, povidone-hydrogen peroxide.

Polyvinylpyrrolidone (PVP, Povidone) is manufactured by BASF Aktiengesellschaft, Unternehemensbereich Feincheme, D-6700 Ludwigshaven, Germany and sold under the trademark **KOLIDON®**. Povidone-iodine products and the preparation of such products are described in U.S. Patents 2,707,701, 2,826,532, and 2,900,305 to Hosmer and Siggia, assigned to GAF Corporation and in a number of GAF Corporation publications; see, e.g. *Tableting with Povidone USP* (1981) and *PVP Polyvinylpyrrolidone* (1982).

There is extensive patent literature on the manufacture and use of various iodine-polymer complexes, exemplary of which are: U.S. Patent No. 3,294,765, Hort, et al, 1966 - manufacture of povidone-iodine complex; U.S. Patent No. 3,468,831, Barabas, et.al., 1969 - graft co-polymers of N-vinyl pyrrolidone; U.S. Patent No. 3,468,832, Barabas, et.al., 1969 - graft co-polymers of N-vinyl pyrrolidone; U.S. Patent No. 3,488,312, Barabas, et. al, 1970 - water-insoluble graft polymer-iodine complexes; U.S. Patent No. 3,689,438, Field, et. al., 1972 - cross-linked polymer-iodine manufacture; U.S. Patent No. 3,907,720, Field, et. al., 1975 - cross-linked polymer-iodine manufacture; U.S. Patent No. 4,017,407, Cantor, et. al., 1977 - solid N-vinyl-2-pyrrolidone polymer carriers for iodine; U.S. Patent No. 4,128,633, Lorenz et al, 1978 - preparation of PVP-I complex; U.S. Patent No. 4,139,688, Dixon, 1979 - cross-linked vinylpyrrolidone; U.S. Patent No. 4,180,633, Dixon, 1979 - cross-linked vinylpyrrolidone; U.S. Patent No. 4,190,718, Lorenz, et.al., 1980 -increasing molecular weight of polyvinylpyrrolidone. The use of povidone-iodine is described in my copending patent Application Nos. 07/577,204, 09041990; 07/795,526, 11211991; 07/846,129, 03031992; 07/577,295, 09041990, 07/878,290, 05041992; 07/878,292, 05041992; 07/753,735, 09031991; 07/753,734, 09031991; 07/753,813, 09031991.

Albumin-iodine, abbreviated hereinafter as "ALB-I", is defined for purposes of this invention as a composition of matter consisting essentially of albumin and iodine, the iodine approximately saturating the binding sites on the albumin. Albumin-iodine treatments and preparation are described in my copending patent Application No. 07/844,241, 03021992. A significant portion of the iodine is available for contact and reaction with biological materials and in an amount such that when the ALB-I is dissolved in aqueous solution there exists an equilibrium condition ALB-I $\rightleftharpoons$ ALB + I, wherein oxidizing iodine is in solution in an amount of at least about 0.0.01 wt percent. The invention, thus encompasses, a process of preparing ALB-I comprising reacting substantially pure albumin, preferrably unsterilized, unstabilized and delipidated albumin, with sufficient iodine-containing reagent to substantially saturate all binding sites thereon

Albumin iodine, "ALB-I", is useful in process in which povidone iodine is useful. In general, a liquid composition that contains bacteria, virus, or other pathogenic organisms can be sterilized by passing it in contact with ALB-I. Thereafter, if it is desired to assure total iodine removal, the solution can be passed

into contact with albumin that is less than saturated with iodine, preferably having no more than a trace of iodine or another iodine absorbing material, such as cross-linked povidone to remove the excess iodine. Likewise a reducing agent such as a reducing sugar, ascorbate, sodium sulfite, etc., may be added to eliminate the last traces of oxidizing iodine. Reducing sugars, ascorbic acid (Vitamin C) and its salts, and sodium sulfite are well-known, readily available reducing agents that are physiologically acceptable. However, any physiologically acceptable reducing agents may be used.

The teachings of the prior art suggest that neither H_2O_2 nor PVP-H_2O_2, would be an effective and reliable biocide in a fluid or in a body, e.g. blood, packed or concentrated cells, organs, etc. in which massive amounts of protein are be available to react with the H_2O_2 it would be expected that both cells and fluids would be detrimentally effected by the strong oxidative power of PVP-H_2O_2. Such use is, contrary to the strong suggestions of the prior art, a central feature of this invention.

One would not consider povidone-hydrogen peroxide as a candidate for killing pathogenic microbes in sperm-carrying liquids because of the oxidative degradation of tissues and cells. Such use is, however, contemplated within the parameters of this invention.

Excess hydrogen peroxide is removed by contacting the material undergoing treatment with solid, e.g. cross-linked, povidone particles or surfaces or neutralize, i.e. converted to water, using any of several physiologically acceptable reducing agents, e.g. vitamin C.

Various medical and blood handling procedures are referred to hereinafter. These are all well-known procedures and steps in these procedures are fully described in the literature. The following references are provided for general background and as sources for detailed reference to the literature as to specific procedures: **TECHNICAL MANUAL of the American Association of Blood Bankers**, 9th Ed. (1985); **HLA TECHNIQUES FOR BLOOD BANKERS**, American Association of Blood Bankers (1984); **Developments in Biological Standardization**, Vols. 1 - 57, S. Karger, Basel; **CLINICAL IMMUNOCHEMISTRY**, The American Association for Clinical Chemistry; **MEDICINE**, Vols. 1 - 2, Scientific American, New York; **Care of the SURGICAL PATIENT**, Vols 1 - 2, Scientific American, New York;

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley-Interscience, John Wiley & Sons, New York.

Summary of the Invention

Povidone (PVP, polyvinyl pyrrolidone) is known to possess a cytophylactic effect upon blood cells, e.g., red blood cells, and upon other cells and tissues. The cytophylactic benefit of povidone and the biocidal effect of hydrogen peroxide, H_2O_2, are combined in the treatment of blood, plasma, serum, packed blood cells, and other blood products and fractions. Povidone-hydrogen peroxide, PVP-H_2O_2, is, of course, known and has been used in methods for sterilizing hard surfaces and in the topical treatment of tissues and membranes.

PVP-H_2O_2 is useful in process in which povidone iodine is useful. In general, a liquid composition that contains bacteria, virus, or other pathogenic organisms can be sterilized by passing it in contact with PVP-H_2O_2. Thereafter, if it is desired to assure total peroxide removal, the solution can be passed into contact with PVP that is less than sanurated with hydrogen peroxide, preferably having no more than a trace of hydrogen peroxide or another hydrogen peroxide absorbing material, such as cross-linked povidone to remove the excess hydrogen peroxide. Likewise a reducing agent such as a reducing sugar, ascorbate, sodium sulfite, etc., may be added to eliminate the last traces of oxidizing hydrogen peroxide. Reducing sugars, ascorbic acid (Vitamin C) and its salts, and sodium sulfite are well-known, readily available reducing agents that are physiologically acceptable. However, any physiologically acceptable reducing agents may be used.

This invention comprises the use of PVP-H_2O_2 for the manufacture of a medicament consisting essentially of blood cells in plasma or another carrier liquid for the treatment of disorders wherein the patient requires the transfusion of blood cells, the PVP-H_2O_2 being added in an amount in excess of that required to kill or inactivate all microbes therein comprising from 0.01w/o to 5w/o of the medicament. Transfusion, transplantation and sperm-containing compositions are similarly prepared.

The invention is embodied in a method of disinfecting biological materials. The steps of the method include treating biological material before

separation of the components thereof with PVP-H_2O_2 to provide from a concentration of 0.01w/o to 5w/o PVP-H_2O_2 in said material before separation of the components thereof. A derivative of the material resulting from the preceding step is prepared and, optionally, also treated with PVP-H_2O_2 to provide from 0.01w/o to 5w/o PVP-H_2O_2 in the derivative. Also optionally the derivative may be treated by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide. These methods are applicable, for example, to whole blood, plasma, tissue, culture nutrient, sperm cells, packed red blood cells and cell-bearing liquids or non-cell-bearing biological liquids.

The invention also includes drug delivery material comprising blood cell concentrate wherein the cell walls of the cells have been opened by treatment with from 0.01w/o to 5w/o PVP-H_2O_2, a drug has been introduced into the cells through passages produced by the PVP-H_2O_2 treatment, the cell walls have been sealed by heating the cells to from 42 to 48 oC. and the resulting material optionally having been treated by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide.

Also included in the invention is the improved method of treating patients with plasma comprising the steps of collecting plasma from a donor, and thereafter infusing the plasma into the patient to be treated, of mixing the plasma with PVP-H_2O_2 sufficient to resulting a PVP-H_2O_2 a concentration of from about 0.01w/o to about 5w/o, and allowing contact of said plasma with said PVP-H_2O_2 for at least about one-half minute sufficient to inactivate or destroy infective pathogenic microbes in the plasma and optionally thereafter removing oxidizing hydrogen peroxide from the resulting mixture by passing said mixture into intimate contact with cross-linked PVP or adding a physiologically acceptable reducing agent.

An apparatus for treatment of liquid to kill microbes is also provided. The apparatus is in the form of a liquid container having, in use an upper reservoir portion for holding said liquid and a lower elutriation portion for recovering liquid and structure defining first and second beds of particulate matter, the first bed comprising substantially insoluble PVP-H_2O_2 and the

second bed consisting essentially of substantially insoluble PVP; the beds being so formed and configured as to permit the passage of the liquid therethrough in intimate contact with the surfaces of the particles forming the respective beds. The first bed may be cross-linked PVP. The apparatus may comprising a third layer between the first and second layers, the third layer comprising substantially insoluble PVP hydrogen peroxide particulate matter. The apparatus may contain a layer of particulate matter comprising an hydrogen peroxide reducing agent. A layer of soluble PVP-H_2O_2 may be provided on the first layer in the liquid reservoir. All or only part of the layers, after the first and second layers, may be provided.

One method of sterilizing an implantable tissue in accordance with this invention comprises placing tissue that is physiologically acceptable for implantation into a human patient into a vacuum chamber, evacuating the chamber and maintaining a vacuum on the chamber for a period long enough to extract at least about one-half of the unbound water originally present in said tissue, and introducing into the vacuum chamber a solution of PVP-H_2O_2 for thereby reconstituting into the tissue said solution in place of the water that was vacuum extracted. Optionally, hydrogen peroxide may be removed by washing or reconstituting the tissue with a reducing agent such as ascorbic acid or a salt thereof or sodium sulfite, for example.

PVP-H_2O_2 may be used alone or in combination with povidone iodine or albumin iodine as disclosed in my copending patent Applications identified hereinbefore. Thus, in one embodiment, the invention comprises a method of disinfecting biological materials comprising the steps of treating biological material before separation of the components thereof with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.01w/o to 5w/o of iodine-containing complex in said material before separation of the components thereof and thereafter preparing a derivative of the material resulting from the preceding step. Optionally, the derivative may be treated with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o

to 10w/o PVP-H_2O_2 and from 0.01w/o to 5w/o of iodine-containing complex in said derivative. As a further option, the derivative may be further treated by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide and iodine, if iodine-containing complex is used.

Brief Description of the Drawings

Figure 1 depicts an apparatus for contacting a liquid material with PVP-H_2O_2 and with either or both of (a) an hydrogen peroxide absorbing material and/or (b) an hydrogen peroxide reducing material, and for providing other materials for processing biological liquids, in particular, according to this invention.

Figure 2 depicts, largely schematically, an apparatus for treating solid tissue samples.

Description of the Preferred Embodiments

Povidone-hydrogen peroxide, abbreviated hereinafter as PVP-H_2O_2, is prepared beginning as described by Garelick et al, supra, or any other convenient method. It is considered most convenient to use solid povidone-hydrogen peroxide as the hydrogen peroxide source because this material provides a convenient source of hydrogen peroxide, has high hydrogen peroxide content and does not add other chemicals or constituents to the resulting PVP-H_2O_2. A bed of solid povidone-hydrogen peroxide, cross-linked povidone reacted with hydrogen peroxide or povidone that has been cross-linked by reaction with hydrogen peroxide that binds to the povidone during cross-linking, or any other solid povidone hydrogen peroxide may be used.

The use of PVP-H_2O_2, optionally followed by treatment with a physiologically acceptable reducing agent for the manufacture of a medicament is contemplated by this invention. Such a medicament may, for example, consist essentially of blood cells in plasma or another carrier liquid. Such medicaments may be used for the treatment of disorders wherein the patient requires the transfusion of blood cells. PVP-H_2O_2 is added in an amount in excess of that required to kill or inactivate all microbes is added. PVP-H_2O_2 may comprise, for example, from about 0.01 to 10 weight percent, preferably from 0.01w/o to 5w/o of the blood. The PVP-H_2O_2 is allowed to remain in

contact with the blood cells or plasma, or other biological material being prepared to be a medicament, for a period of at least about a half a minute sufficient to kill the microbes, but under about 24 hours, at low concentrations, and 1 hour at higher concentrations, so as not to denature or otherwise injure the biological material. Usually, contact of under an hour is preferred. Accordingly, the contact times will be referred to as from one-half minute to one hour with the caveat that longer contact is not necessary or beneficial and may result in injury to the biological, but would, nevertheless, be within the scope of the invention. The reducing agent is then added in an amount to reduce substantially all hydrogen peroxide. The maximum amount of reducing agent required is easily calculated. The actual amount normally required, to which a safety margin amount will be added, is determined by an hydrogen peroxide assay on typical batches using known, routine procedures. A second treatment as described may be performed to assure total sterilization, if desired. Likewise, a second similar treatment may be performed on a product or fraction of the initial biological material treated as described above.

The use of PVP-H_2O_2 and a physiologically acceptable hydrogen peroxide absorbent material, e.g., solid povidone or cross-linked povidone, for the manufacture of a medicament is contemplated by this invention. Such a medicament may, for example, consist essentially of blood cells in plasma or another carrier liquid. Such medicaments may be used for the treatment of disorders wherein the patient requires the transfusion of blood cells. Either simultaneously therewith, or afterward, PVP-H_2O_2 in an amount in excess of that required to kill or inactivate all microbes is added. PVP-H_2O_2 may comprise, for example, from about 0.01 to 10 weight percent, preferably from 0.01w/o to 5w/o of the medicament. The PVP-H_2O_2 is allowed to remain in contact with the blood cells or plasma, or other biological material being prepared to be a medicament, for a period of at least about a half a minute sufficient to kill the microbes, but not long enough to denature or otherwise injure the biological material. Usually, contact of under an hour is preferred. Accordingly, the contact times will be referred to as from one-half minute to one hour with the caveat that longer contact is not necessary or beneficial and may result in injury to the biological, but would, nevertheless, be within the

scope of the invention. The mixture resulting from the above is then contacted with an hydrogen peroxide absorbing reagent such as cross-linked PVP, or povidone, to remove the hydrogen peroxide. If desired, a reducing agent may thereafter be added in an amount to reduce any hydrogen peroxide that may not have been absorbed. The contact with the hydrogen peroxide absorbing material is preferably accomplished by passing the material undergoing treatment through a layer, i.e. a bed or filter, of solid, substantially insoluble povidone. A second treatment as described may be performed to assure total sterilization, if desired. Likewise, a second similar treatment may be performed on a product or fraction of the initial biological material treated as described above.

In a similar manner, the "addition" of a reducing agent to the material undergoing treatment may be accomplished by passing the material through a layer of substantially insoluble material that has active reducing sites thereon or equilibrates with the liquid material undergoing treatment to partially dissolve into such liquid, or make readily available in said liquid (as by swelling, for example) reducing moieties. A bed of beads or fibers, for example, that expose on the surface thereof reducing sugar moieties may be used very conveniently.

Reference is made to Figure 1 of the drawing for a better understanding of the invention in one form. Figure 1 depicts an apparatus for contacting a liquid material with PVP-H_2O_2 and with either or both of (a) an hydrogen peroxide absorbing material and/or (b) an hydrogen peroxide reducing material, and for providing other materials for processing biological liquids, in particular, according to this invention. The apparatus, being shown and described in a generally schematic fashion, may be in any of many configurations. The only significant structure, insofar as this invention relates is to the arrangement of the layers

The apparatus 10 may be viewed as a filter funnel or a column. As those in the art understand, the difference between a filter and a column is often insignificant in that both "filter" a liquid and both cause the liquid to contact solid material. A filter may, indeed must, remove only part of the material. For example, either a filter or a column may let small cells or particles pass but retain larger cells, or it may permit only liquid and extremely

small particles pass. The apparatus comprises cylindrical portion 12 that, in part, defines a reservoir portion. The reservoir may be large or very small as desired. The apparatus, in the configuration depicted comprises a second, smaller cylindrical tube portion 14 and a conical transition zone 16 connecting the two cylindrical portions as is conventional in funnel manufacture. It is again emphasized, however, that it is immaterial whether the apparatus defines a reservoir and or funnel portion of any particular size or configuration.

The apparatus defines a first layer 20 and a second layer 22. The first layer is made up of substantially insoluble PVP-H_2O_2. This layer is described as being made up of particulate materials in that the use or particulates in one way or another is usually involved. Particles of solid, insoluble PVP-H_2O_2, e.g. cross-linked PVP-H_2O_2, in the form of a layer or bed of particles, either supported directly by a layer below or by way of another support, e.g. being bonded to or entrapped within a layer of fibers or particles, is contemplated. The first layer may also contain some soluble PVP-H_2O_2. A frit made of particles bound together adhesively, by heat or pressure would also be within the disclosure and invention. The PVP-H_2O_2 may be formed *in situ* by reacting a layer of povidone with hydrogen peroxide or the layer may be made up of pre-synthesized PVP-H_2O_2.

The second layer is downstream of the first layer, i.e. the liquid to be treated flows through the first layer and then the second layer. The second layer may comprise an insoluble hydrogen peroxide absorbent, e.g. cross-linked povidone, or insolublized povidone, or an hydrogen peroxide reducing agent, or a mixture of both, or be a multiple sub-layer structure with a sublayer of hydrogen peroxide absorbent first and then a sublayer of hydrogen peroxide reductant. Again, the layer may be a self-supporting frit or other structure or may be supported by a support or other layer.

The essential function of the apparatus is to cause a liquid that is to be treated to pass, with or without cells or other particles therein, first through a layer of PVP-H_2O_2 and, thereafter, to contact such liquid with absorbent to remove the hydrogen peroxide and/or reductant to reduce the hydrogen peroxide. Hence, the layers may be quite deep or quite thin, adjacent each

other or spaced from each other, as is necessary or desirable to provide adequate contact of the liquid with each of the layers or beds.

Such an apparatus is conveniently suited for the treatment of liquid to kill microbes in the liquid. The liquid container that is generally defined by the overall apparatus in the simplified, schematic example of Figure 1, and has an upper or liquid inflow reservoir portion for holding liquid to be treated. This may be a very small reservoir or quite large. The reservoir may displaced from the beds or layers by a very large distance, though this is not generally beneficial. The apparatus has a lower or elutriation or recovery portion for recovering liquid that has been treated. Between these portions, first and second beds of particulate matter are defined by suitable structure. The first bed or layer comprises substantially insoluble PVP-H_2O_2. The second bed consists essentially of substantially insoluble povidone, or other hydrogen peroxide absorbent, and/or hydrogen peroxide reducing agent. The beds are so formed and configured as to permit the passage of the liquid therethrough in intimate contact with the surfaces of the particles forming the respective beds. The usual and most common hydrogen peroxide absorbent is cross-linked povidone.

The apparatus may desirably further comprise a third layer 24 between the first and second layers. The third layer comprises substantially insoluble povidone hydrogen peroxide particulate matter. The presence of the third layer entraps and regenerates hydrogen peroxide and significantly increases the biocidal activity of hydrogen peroxide.

A fourth layer 26, which may be in the form of a sublayer within the second layer, comprising particulate hydrogen peroxide reducing agent may be provided downstream from the second layer to provide for the reduction of any residual hydrogen peroxide from I2 to iodide, or, if reduction is earlier provided, to add a safety step to assure that all oxidizing hydrogen peroxide has been reduced.

In many applications, it may be desirable to provide a fifth layer 28 of soluble PVP-H_2O_2 on the first layer in the liquid reservoir to permit the actual dissolution into the liquid of substantial amounts of PVP-H_2O_2 and thereby provide a greater reservoir of more available hydrogen peroxide to the liquid

The fifth layer may also comprise soluble povidone, preferably low molecular weight (MW < 12,000 daltons) on the top of the first layer for being dissolved into the liquid to be treated to provide a cell protective milieu for the cells carried by the liquid. In like manner, the fifth layer may comprise soluble PVP-H_2O_2 to provide both hydrogen peroxide and cell protection. Preferably, at least about one-fourth of all the PVP in solution is low molecular weight PVP, i.e., MW < ~ 15,000.

The first and second layers are essential to the full and proper functioning of the apparatus. After those layers or beds, however, any number of additional layers or additives may be provided, so long as they do not interfere with the combined function of the first and second beds or layers.

All of the layers just described may, conveniently but not necessarily, be supported by a layer 30 that may be a frit, a filter paper or a porous layer. The thickness of the beds may be the same or greatly different. It is a simple matter to calculate contact time in a column and to provide suitable beds of materials therein.

Any of the beds may be made up the active material, e.g. PVP-H_2O_2, reducing sugar, etc., attached to carrier particles, such as ground glass, charcoal, ion exchange resin, cellulose derivatives, etc. The particulate matter may, in a preferred form, consist essentially of particles having a diameter of from about 10 to about 100 microns, but any size that permits suitable flow rates and assures intimate contact may be used.

The use of PVP-H_2O_2 and a physiologically acceptable reducing agent for the manufacture of transfusion biological material from one human or mammal for transfusion of such material to another human or mammal, or the transplant or transfusion biological material is a part of this invention. The transfusion or transplant is disinfected with a PVP-H_2O_2 solution having concentration of from about 0.01 to 10 weight percent, preferably 0.01w/o to 5w/o, and thereafter treated with the reducing agent to reduce the residual hydrogen peroxide. Liquid materials may be treated in any suitable manner, such as has been described. Solid tissue samples may be treated simply be soaking, by infusing or by vacuum infusing.

Figure 2 depicts, largely schematically, an apparatus for treating solid tissue samples. The apparatus comprises a chamber system 100 capable of withstanding the forces of a vacuum. In the merely exemplary form shown, a cylinder 102 is closed at the respective ends by end covers 104 and 106, the end 106 being removable to gain access to the inside of the chamber. For example, a portion 108 of the end 106 may be slipped into the cylinder 102 and sealed using "O" rings, etc., to provide a vacuum tight seal. A vacuum line 110 through valve 112 and line 114 permits evacuation of the chamber. An input line 120, coupled to valve 122 and line 124 permits the introduction of liquid into the chamber. A platform 126, secured to the end 106, supports a tissue sample 130. The tissue sample is placed in the chamber, the chamber evacuated and then liquid is introduced, thereby substantially replacing water in the sample with the liquid introduced.

Implantable tissues may be treated to kill microbes, i.e. "sterilized" by placing tissue that is physiologically acceptable for implantation into a human patient into a vacuum chamber, evacuating the chamber and maintaining a vacuum for a period long enough to extract at least about one-half of the unbound water originally present in said tissue and then introducing into said vacuum chamber a solution of PVP-H_2O_2 for thereby reconstituting into the tissue said solution in place of the water that was vacuum extracted. The thus treated tissue may then be soaked in a solution of an physiologically acceptable hydrogen peroxide reducing agent. Alternatively, the chamber may again be evacuated to extract the PVP-H_2O_2 solution from the tissue and a solution of physiologically acceptable hydrogen peroxide reducing agent introduced into the vacuum chamber for saturating the tissue for reducing any residual hydrogen peroxide.

As a method of disinfecting blood derivatives, the invention may comprise treating blood before separation of the components thereof with PVP-H_2O_2 to provide from a concentration of from about 0.01 to 10 weight percent, preferably 0.01w/o to 5w/o, PVP-H_2O_2 in the blood, preparing a derivative of the blood from step, treating the derivative with PVP-H_2O_2 to provide from about 0.01 to 10 weight percent, preferably 0.01w/o to 5w/o, hydrogen peroxide in the derivative thereafter treating the derivative by addition of a

physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide.

It is believed that PVP-H_2O_2 opens pathways through the cell wall which permits certain components of the cell, e.g. potassium salts, to "leak" from the cell. By the same mechanism, treatment of red blood cells with from one to about five percent hydrogen peroxide as PVP-H_2O_2 opens the cells to "inward leaking". Thus, compounds which have a virucidal or other effect in the cell can be introduced into the cell. PVP-H_2O_2 can, for example, be used as described to increase the uptake of antiviral compounds, e.g. carbenoxolone, AZT, etc., which, in turn, may prevent the replication of virus in the cell. The net effect of this procedure is a biological synergism. A new drug delivery system involves the use of PVP-H_2O_2 to open pathways through the cell wall of red blood cells. Red blood cell concentrates are treated as described to open passageways into the cell. The then permeable cell is emersed in or treated with a drug which is to be delivered to the patient. The cell walls having passages therethrough permit the drug to enter into the cell. Thereafter, the hydrogen peroxide may be removed and the cell concentrate is heated to 42 - 48 oC to seal the cell walls. The concentrated cells are then infused into the patient where they carry out the normal function of such cells. These cells have a finite life. As the cells age, they lyse, thereby releasing the drug directly into the blood stream where the drug can become effective.

Infective pathogenic microorganisms are believed to be inactivated when PVP-H_2O_2 is used in solution to perfuse tissues and organs after removal from the donor and before transplantation to the recipient. The perfusion solution contains molecular hydrogen peroxide compound in a concentration of from about 0.01 to 10 weight percent, preferably 0.01w/o to about 5w/o (10 to 5000 ppm I2), preferably from about 0.25%/o to about 2%/o. After a period of time, most of the unreacted molecular hydrogen peroxide compound is washed away and any residual molecular hydrogen peroxide compound is absorbed into the protein or converted to inactive iodides, e.g. using ascorbate or other reducing agent as described, and does not significantly interfere with acceptance by the recipient.

Sperm-bearing solutions treated to be made freed of pathogenic microbes by washing and/or storing the sperm in a solution which contains a concentration of PVP-H_2O_2in a concentration is from about 0.01 to 10 weight percent, preferably 0.01w/o to about 5w/o, (100 to 5000 ppm I2) and, preferably, sufficient to inactivate bacteria, viruses and other pathogenic organisms, and washing the sperm cells in the solution, optionally with a solution of a reducing agent compound.

PVP-H_2O_2 is considered to be effective in protecting the sperm cells from the spermicidal activity of hydrogen peroxide sufficient to permit the killing of pathogenic organisms while leaving viable, motile sperm cells suitable for artificial insemination. The washing is continued or repeated to assure that substantially all of the seminal fluid is replaced with PVP-H_2O_2 solution. Other reagents such as are conventionally used in sperm treatment, storage and preparation, or for particular purposes may, of course, also be included in the infusion solution. If desired, residual hydrogen peroxide may be washed out and/or removed using ascorbate or other reducing agent and any suitable storage fluid, including solutions of polyvinyl pyrrolidone, may be used to store and handle the sperm cells.

The above applications in which the material to be purified is a liquid or cells carried in a liquid can be carried out by flowing the liquid through a bed (e.g. the conventional filter structure of solid particles on a porous or foraminous support) of solid particles of PVP-H_2O_2 of suitable size or by contacting the liquid and/or the cells in the liquid with particles or a membrane or surface of solid PVP-H_2O_2. Where a bed of particles is used with a cell-bearing liquid, the particles must be large enough to permit intimate contact without entrapping or binding the cells. The liquid may then be passed through a layer or in contact with solid phase PVP-H_2O_2 to assure complete biocidal effect. Thereafter, the liquid is passed through or into intimate contact with cross-linked PVP to absorb the molecular hydrogen peroxide from the liquid. Finally, a reducing agent such as ascorbate may be added if considered necessary as a precaution.

In carrying out this facet of the invention, the liquid or cell-bearing liquid is contacted with the solid PVP-H_2O_2. This may be done most

efficiently, in most cases, by passing the liquid through a settled or fluidized or packed bed of PVP-H_2O_2 particles; however, such approaches will not, ordinarily, be suitable for treating cell-bearing liquids. Cell-bearingliquids may be treated by mixing the particles in a container of the liquid or passing the liquid over a surface of the PVP-H_2O_2 material, e.g. over a multiple-plate array of sheets of such material. The PVP-H_2O_2 may be washed and the hydrogen peroxide content therein regenerated between uses.

In general a solution of reducing agent , e.g. a reducing sugar (or mixtures of reducing sugars), ascorbic acid or ascorbate, a sulfite, e.g. sodium sulfite, etc. in which the agent is in a concentration of 0.01 to 1 percent is suitable and such is implicit unless otherwise noted.

In all of the above methods, the invention may be carried using PVP-H_2O_2 as described, alone, or in combination with PVP-I or ALB-I, or combinations of PVP-I and ALB-I, the latter being in concentrations of from 0.001 to 5w/o.

Industrial Application

This invention finds application in medicine and veterinary science.

WHAT IS CLAIMED IS:

1. The use of PVP-H_2O_2 for the manufacture of a medicament consisting essentially of blood cells in plasma or another carrier liquid for the treatment of disorders wherein the patient requires the transfusion of blood cells, the PVP-H_2O_2 being added in an amount in excess of that required to kill or inactivate all microbes therein comprising from 0.01w/o to 10w/o of the medicament.

2. The use of PVP-H_2O_2 and a physiologically acceptable reducing agent in the manufacture of sperm cell-containing compositions for the induction of pregnancy in a female by inseminating the sperm cells into the female, the sperm cells being washed with PVP-H_2O_2 in water solution in a concentration of from 0.01w/o to 10w/o, sufficient to kill bacteria, viruses and other pathogenic micro-organisms but insufficient to inactivate the sperm cells and optionally adding said reducing agent in an amount to reduce substantially all of the hydrogen peroxide.

3. The use of PVP-H_2O_2 and a physiologically acceptable reducing agent for the manufacture of transfusion biological material from one human or mammal for transfusion of such material to another human or mammal, the transplant or transfusion biological material being disinfected with a PVP-H_2O_2 solution having concentration of from 0.01w/o to 10w/o and thereafter adding said reducing agent to reduce the residual hydrogen peroxide.

4. A method of disinfecting biological materials comprising the steps of:

(a) treating biological material before separation of the components thereof for from about one minute to about one hour with PVP-H_2O_2 to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 in said material before separation of the components thereof;

(b) preparing a derivative of the material resulting from step (a);

(c) treating said derivative with PVP-H_2O_2 to provide from 0.01w/o to 10w/o PVP-H_2O_2 in the derivative; and optionally

(d) treating the derivative by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide.

5. The method of Claim 4 wherein the biological material is whole blood.

6. The method of Claim 4 wherein the biological material is blood plasma.

7. The method of Claim 4 wherein the biological material is body tissue.

8. The method of Claim 4 wherein the biological material is tissue culture nutrient.

9. The method of Claim 4 wherein the biological material is packed red blood cells.

10. The method of Claim 4 wherein the biological material is a cell bearing liquid.

11. In the method of treating patients with plasma comprising the steps of collecting plasma from a donor, and thereafter infusing the plasma into the patient to be treated, the improvement comprising the additional steps of: mixing the plasma with PVP-H_2O_2 sufficient to resulting a PVP-H_2O_2 a concentration of from about 0.01w/o to about 10w/o, and allowing contact of said plasma with said PVP-H_2O_2 for at least about one-half minute sufficient to inactivate or destroy infective pathogenic microbes in the plasma and optionally thereafter removing oxidizing hydrogen peroxide from the resulting mixture by passing said mixture into intimate contact with cross-linked PVP or povidone or adding a physiologically acceptable reducing agent.

12. The use of PVP-H_2O_2 and a physiologically acceptable reducing agent for the manufacture of transfusion biological material from one human or mammal for transfusion of such material to another human or mammal, the transplant or transfusion biological material being disinfected with a PVP-H_2O_2 solution having concentration of from 0.01w/o to 10w/o and optionally thereafter adding said reducing agent to reduce the residual hydrogen peroxide.

13. An apparatus for treatment of liquid to kill microbes therein comprising a liquid container having, in use an upper reservoir portion for holding said liquid and a lower elutriation portion for recovering liquid and structure defining first and second beds of particulate matter, the first bed comprising substantially insoluble PVP-H_2O_2 and the second bed consisting

essentially of substantially insoluble PVP or povidone; the beds being so formed and configured as to permit the passage of the liquid therethrough in intimate contact with the surfaces of the particles forming the respective beds.

14. The apparatus of Claim 13 wherein the substantially insoluble PVP is cross-linked PVP.

15. The apparatus of Claim 13 further comprising an additional layer between the first and second layers, said additional layer comprising substantially insoluble PVP hydrogen peroxide particulate matter.

16. The apparatus of Claim 13 further comprising an additional layer of particulate matter below the second layer, said additional layer comprising an hydrogen peroxide reducing agent.

17. The apparatus of Claim 13 further comprising an additional layer of soluble PVP-H_2O_2 on the first layer in the liquid reservoir.

18. The apparatus of Claim 13 wherein the insoluble PVP-H_2O_2 particles of the first layer are physically supported by a layer of fibrous material.

19. A method of sterilizing an implantable tissue comprising:

(a) placing tissue that is physiologically acceptable for implantation into a human patient into a vacuum chamber;

(b) evacuating said chamber and maintaining a vacuum on said chamber for a period long enough to extract at least about one-half of the unbound water originally present in said tissue; and

(c) introducing into said vacuum chamber a solution of PVP-H_2O_2 for thereby reconstituting into the tissue said solution in place of the water that was vacuum extracted;

20. The method of Claim 19 further comprising the step of soaking the thus treated tissue in a solution of an physiologically acceptable hydrogen peroxide reducing agent.

21. The method of Claim 20 further comprising the following steps after said step (c):

(d) evacuating the chamber to extract the PVP-H_2O_2 solution; and

(e) introducing a solution of physiologically acceptable hydrogen peroxide reducing agent into the vacuum chamber for saturating the tissue with said solution for reducing any residual hydrogen peroxide.

22. A method of disinfecting biological materials comprising the steps of:

(a) treating biological material before separation of the components thereof for a period of from about one minute to about one hour with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.001w/o to 5w/o of iodine-containing complex in said material before separation of the components thereof;

(b) optionally preparing a derivative of the material resulting from step (a); and optionally treating said derivative with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.01w/o to 5w/o of iodine-containing complex in said derivative; and optionally

(c) treating the biological material or derivative thereof by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide and iodine.

23. The method of Claim 22 wherein the biological material is whole blood.

24. The method of Claim 22 wherein the biological material is blood plasma.

25. The method of Claim 22 wherein the biological material is body tissue.

26. The method of Claim 22 wherein the biological material is tissue culture nutrient.

27. The method of Claim 22 wherein the biological material is packed red blood cells.

28. The method of Claim 22 wherein the biological material is a cell bearing liquid.

29. A method of disinfecting biological materials comprising the steps of:

(a) treating biological material before separation of the components thereof with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.001w/o to 5w/o of iodine-containing complex in said material before separation of the components thereof;

(b) optionally preparing a derivative of the material resulting from step (a); and optinally treating said derivative with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.01w/o to 5w/o of iodine-containing complex in said derivative; and

(c) treating the derivative by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide and iodine, if iodine-containing complex is used.

30. The method of Claim 29 wherein the biological material is whole blood.

31. The method of Claim 29 wherein the biological material is blood plasma.

32. The method of Claim 29 wherein the biological material is body tissue.

33. The method of Claim 29 wherein the biological material is tissue culture nutrient.

34. The method of Claim 29 wherein the biological material is packed red blood cells.

35. The method of Claim 29 wherein the biological material is a cell bearing liquid.

36. A method of disinfecting biological materials comprising the steps of:

(a) treating biological material before separation of the components thereof with PVP-H_2O_2 and an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.001w/o to 5w/o of iodine-containing complex in said material before separation of the components thereof;

(b) preparing a derivative of the material resulting from step (a);

(c) optionally treating said derivative with PVP-H_2O_2 and optionally an iodine-containing complex selected from the group consisting of povidone-iodine or albumin-iodine or mixtures thereof to provide from a concentration of 0.01w/o to 10w/o PVP-H_2O_2 and from 0.001w/o to 5w/o of iodine-containing complex in said derivative; and

(d) treating the derivative by addition of a physiologically acceptable reducing agent or contact with cross-linked PVP to reduce or remove residual hydrogen peroxide and iodine, if iodine-containing complex is used.

37. The method of Claim 36 wherein the biological material is whole blood.

38. The method of Claim 36 wherein the biological material is blood plasma.

39. The method of Claim 36 wherein the biological material is body tissue.

40. The method of Claim 36 wherein the biological material is tissue culture nutrient.

41. The method of Claim 36 wherein the biological material is packed red blood cells.

42. The method of Claim 36 wherein the biological material is a cell bearing liquid.

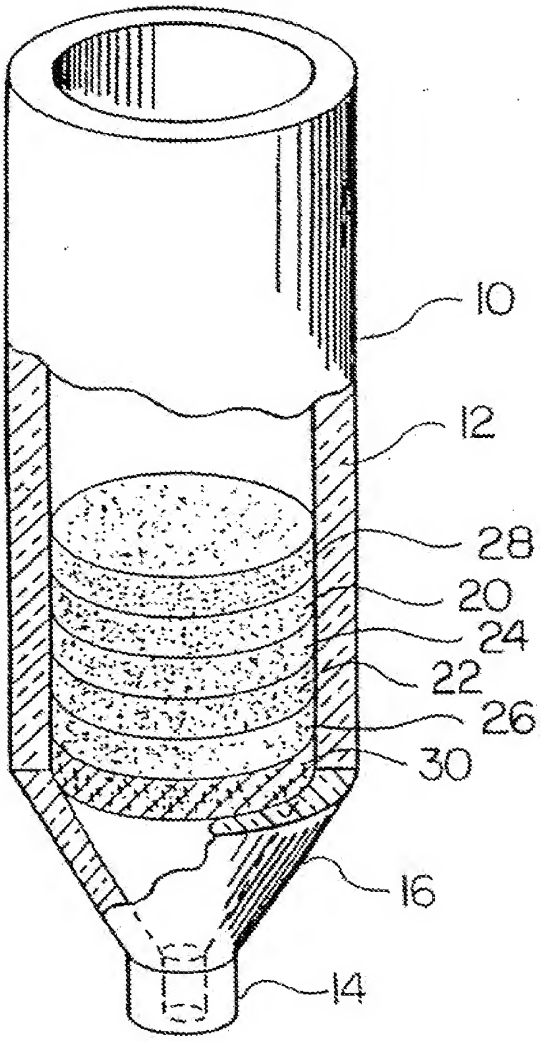
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FIG - 1

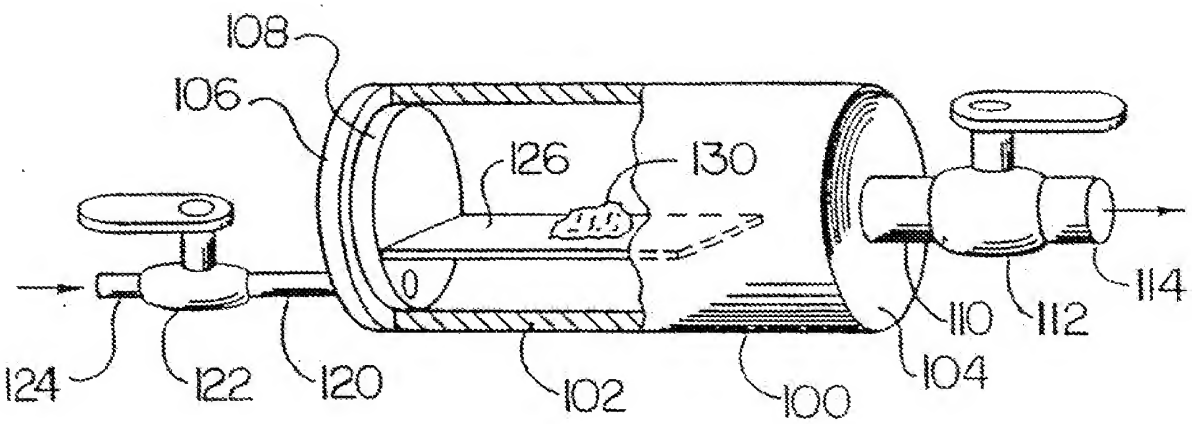
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FIG - 2

INTERNATI· ·EARCH REPORT

International application No.
PCT/US93/06096

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61L 2/18
US CL :422/28, 30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/28, 30

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,065,139 (ERICSSON ET AL) 20 NOVEMBER 1962, See column 2.	4-11, 13-42
Y	US, A, 4,368,081 (HATA ET AL) 11 JANUARY 1983, See column 3.	4-11, 13-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 03 SEPTEMBER 1993	Date of mailing of the international search report NOV 26 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer PETER KULKOSKY Telephone No. (703) 308-2351

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